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Abstract: Genotoxic chemicals can damage the genetic material of humans as well as that of organisms living in the environment. With respect to adverse effects, alterations induced in the germ line, leading to alterations in the genetic make-up of populations, are of primary concern in ecosystems, because somatic changes, even if they lead to a loss of individuals, will not be critical in populations with a large reproductive surplus. This is different in human toxicology where genetic alterations in germ cells as well as in somatic cells of any individual are of concern. Increased frequencies of mutations and related genetic alterations in the gene pools of individual species or populations in ecosystems have to be judged against the background of spontaneous mutations that have enabled species to survive and adapt in changing environments since the beginning of life on our planet, and which have played an important role as the substrate for evolutionary developments. Examples of the selection of altered phenotypes (and genotypes) in response to environmental pollution and environmental stress are melanism in moth populations, metal resistance in plants, insecticide resistance in insects and malaria resistance in humans. Pollution, in general, can represent a stress factor selectively leading to a change in genetic make-up. In addition, environmental genotoxins can directly alter gene pools. A change in the genetic constitution may be advantageous for certain populations living in stressful conditions, but may present a disadvantage for others, including man. Examples are (i) the induction of (pesticide) resistance, (ii) the increased virulence of pathogens, (iii) alterations of host ranges of pathogenic forms or the appearance of new virus types and (iv) subtle changes in parasite—host or predator—prey relationships. Basically the release of genotoxins into the environment should be avoided because massive exposures may affect the reproductive capacity of many species, and modest exposures may lead to an enhanced instability of ecosystems and may provoke specific adaptations to stressful situations. Furthermore, the uncontrolled presence of genotoxins in any compartment of the natural environment is an unwanted situation, in particular also from a human point of view. In addition we need novel quantitative approaches in order to make quantitative risk estimates possible

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DISCUSSION FORUM

Environmental effects of genotoxins (eco-genotoxicology)

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Genotoxic chemicals can damage the genetic material of humans as well as that of organisms living in the environment. With respect to adverse effects, alterations induced in the germ line, leading to alterations in the genetic make-up of populations, are of primary concern in ecosystems, because somatic changes, even if they lead to a loss of individuals, will not be critical in populations with a large reproductive surplus. This is different in human toxicology where genetic alterations in germ cells as well as in somatic cells of any individual are of concern. Increased frequencies of mutations and related genetic alterations in the gene pools of individual species or populations in ecosystems have to be judged against the background of spontaneous mutations that have enabled species to survive and adapt in changing environments since the beginning of life on our planet, and which have played an important role as the substrate for evolutionary developments. Examples of the selection of altered phenotypes (and genotypes) in response to environmental pollution and environmental stress are melanism in moth populations, metal resistance in plants, insecticide resistance in insects and malaria resistance in humans. Pollution, in general, can represent a stress factor selectively leading to a change in genetic make-up. In addition, environmental genotoxins can directly alter gene pools. A change in the genetic constitution may be advantageous for certain populations living in stressful conditions, but may present a disadvantage for others, including man. Examples are (i) the induction of (pesticide) resistance, (ii) the increased virulence of pathogens, (iii) alterations of host ranges of pathogenic forms or the appearance of new virus types and (iv) subtle changes in parasite–host or predator–prey relationships. Basically the release of genotoxins into the environment should be avoided because massive exposures may affect the reproductive capacity of many species, and modest exposures may lead to an enhanced instability of ecosystems and may provoke specific adaptations to stressful situations. Furthermore, the uncontrolled presence of genotoxins in any compartment of the natural environment is an unwanted situation, in particular also from a human point of view. In addition we need novel quantitative approaches in order to make quantitative risk estimates possible.

Introduction

By definition, genotoxins are chemical and physical agents capable of inducing mutations and related genetic changes in living cells of living organisms. As far as multicellular organisms are concerned, we have to distinguish between mutations induced in somatic cells and those induced in germ cells. Mutations occurring in germ cells may enter into the gene pool of the species of concern. Studies on mutagenic chemicals in the environment have almost exclusively focused on potential harmful effects in humans. Very few authors have addressed the potential consequences of exposure to environmental genotoxins of other species living in terrestrial, freshwater or marine environments. Among these are Seiler (1982) and de Raat *et al.* (1985). In this paper it is our objective to address this question again and to place it in a somewhat broader context.

Genetic effects in human toxicology

In human toxicology (or in mammalian model systems used to assess damage to humans) both germinal and somatic mutations are of importance. Somatic mutations and DNA rearrangements (e.g. gene conversion, reciprocal recombination and chromosome aberrations) may contribute to cancer induction, promotion and progression, e.g. by changing the activity of oncogenes (see, e.g. Fearon and Vogelstein, 1990; Würgler, 1992). Their relationship to other degenerative diseases such as certain types of cardiovascular disease (Mulkvag *et al.*, 1988), including atherosclerosis (Penn *et al.*, 1986; Bridges *et al.*, 1990), autoimmune defects and certain types of diabetes (Karsten and Kryspin-Sorensen, 1988) and ageing (Kirkwood, 1989; Slagboom, 1990), is subject to research efforts as well as speculation.

Mutations in germ cells may lead to abortions and may, in this way, reduce fertility. If a new mutation is transmitted to liveborn offspring and does not drastically reduce reproductive fitness, it may enter the human gene pool. Depending on its phenotypic effects in heterozygous carriers, it may persist for a few or many generations and will contribute to the genetic load and related genetic disease in the human population [estimated average persistence: autosomal dominant or X-linked mutations: five generations; recessive mutations: many generations; mutations of complex inheritance: ten generations; structural chromosomal changes, balanced: five, unbalanced: three generations; numerical chromosomal changes: one generation (ICPEMC, 1983)].

Human toxicology versus ecotoxicology: different policy objectives

From the point of view of risk management, the potential effects of an increased frequency of mutations have to be considered differently in humans and in other organisms in our environment. In humans our concern is the potential impairment of health in any *individual* resulting from inherited or somatically acquired mutations. Thus, even a rare event is unwanted. In addition, there is usually only weak selection against mutant genotypes. In ecotoxicology our concern is rather the maintenance of the integrity of ecosystems, which depends on surviving and reproducing *populations*. Usually there is strong selection against phenotypically deviating (less 'fit' or less well-adapted) individuals, coupled with a large reproduction surplus, resulting in a rapid disappearance of harmful mutations. These considerations provide an *a priori* reason to be less concerned about an enhanced mutation frequency in a natural population than in man, as induced by environmental agents. Only in the case of some

larger mammals (seals, whales, elephants, etc.) or birds whose populations are at minimal sizes and which, unlike most plants and insects and other biota in ecosystems, do not have a large reproductive surplus as a survival strategy, may protection at the individual level become an option of conservation.

One might argue that not merely the maintenance of a population but also the conservation of a certain genetic make-up of this natural population is the objective. Here, one should realize that selection by environmental stresses (both of natural origin and man-made) is likely to be much more effective in changing the genetic constitution of a population than even a very high level of mutagen exposure could ever be (see below). Thus, the maintenance of existing genetic composition can hardly be taken as a serious policy goal.

Nevertheless, we will consider more closely whether some types of mutations induced in natural populations might be of relevance in the context of nature conservation. Before addressing this question, however, some remarks should be made about the role that mutation and other genetic changes have always had in the dynamics of biological species.

Mutation, selection and evolution

The history of life on earth has witnessed a continuously changing wealth of different life forms, species and varieties. A crucial constituent of this phenomenon called evolution is the availability of genetic variation within a given species or population. This variation enables a population to cope with variations of its environmental conditions, both in space and time. Known mechanisms in the production of this genetic variation (in higher organisms) include, apart from meiotic recombination (in sexual reproduction), mutation, chromosome rearrangement, mitotic recombination, transposon activity and gene amplification. These processes occur spontaneously and are considered random, in most cases, with respect to their effect, i.e. not directed by the environmental requirements. Yet, in some organisms there is evidence for enhanced mutation frequencies occurring under unusual stress conditions (see below) and gene amplification can be directed towards a desired function (e.g. amplification of a metallothionein gene to confer metal resistance). In other words, mutations and other genetic changes of the same types that can be induced by genotoxic chemicals are a necessary ingredient of the process of maintaining life on earth.

As most genetic changes are random, it is clear that the great majority will be harmful or at best neutral and only a few will present an improvement given the actual environmental condition. That is, the process of providing the necessary genetic variation is not without cost. This cost is the proportion of individuals carrying harmful mutations that are thus eliminated rapidly from the population (Dickerson and Geis, 1969; Wilson *et al.*, 1977; Watson *et al.*, 1981). Only neutral or beneficial mutations are expected to persist. The study of protein evolution from bacteria to man has shown that, depending on the functional requirements of the protein, mutational changes are eliminated at different rates, or are 'tolerated' to different degrees, also dependent on the precise position within the protein. This leads to a balance between spontaneous mutagenesis creating new variants and the constantly changing environmental factors determining the persistence of such variants (reviewed by Crow and Simmons, 1983).

The theory of population genetics describes the frequencies of different genetic variants within populations and the changes in these frequencies as a consequence of the selective advantages or disadvantages of the variants to their carriers, in probabilistic terms. Of importance is the fact that selection acts on phenotypes, whereas the genotypes determine the contribution of certain variants to the next generation. In this sense, a population is both a collection of genetically heterogeneous individuals and a collective reservoir of genetic information. A key concept is that of (Darwinian) fitness, which can be defined as the relative probability of a given phenotype to transmit its genes to the next generation, under a given set of environmental conditions.

Within the present context, two aspects are of importance: (i) mutation is one of the mechanisms that maintain genetic variation within a population and thus enables that population to cope with changing and sometimes adverse environmental circumstances, and (ii) this happens at the cost of the production of a proportion of genetically less fit zygotes, which normally does not present a threat to the maintenance of the populations.

Environmental stress and changes in a population's genetic constitution

In specific environmental stress situations, selection may favour genetic variants that were not advantageous before and thus may lead to substantial changes in the genetic make-up of the population. The literature provides many examples of such adaptations, the environmental stress being either of natural or man-made origin. Examples of stresses of natural origin include the appearance of climatic races of *Drosophila melanogaster* during periods of short-term high- and low-temperature stress (Parsons, 1980, 1983). In general the variability of quantitative characteristics tends to be high under conditions of severe environmental stress (Parsons, 1983). This conclusion applies directly to quantitative traits important in determining survival and necessarily less directly to genes controlling, for example, household proteins. Another example, the appearance of a wingless mutation in a fly population on an island with strong winds (as found on the Atlantic island Tristan da Cunha), where these flies have an advantage over those with normal wings, was observed. In populations with a lower turnover environmental stress may narrow down the genetic diversity by killing the more sensitive variants. This phenomenon has also been described for populations of forest trees suffering from air pollution (Scholz *et al.*, 1989). Examples of adaptations or unexplained genetic changes in response to man-made environmental stress are numerous. Certain marine animals exposed to thermal pollution showed changed allozyme frequencies (Mitton and Koehn, 1975; Nevo *et al.*, 1977, 1978; Battaglia and Beardmore, 1978). Environmental selection has been demonstrated not only for temperature, but also for other factors, including salinity and pressure in the ocean (Siebenaller and Somero, 1978; Koehn *et al.*, 1980; Watt, 1983; Mueller *et al.*, 1985). Using laboratory-grown populations, Battaglia *et al.* (1980) demonstrated changes in the genetic constitution of the copepod *Tisbe* in response to toxicant exposure (Bayne *et al.*, 1985). One of the very first recorded effects of pollution on wildlife, and perhaps the most striking evolutionary change ever to be actually witnessed, was the occurrence of melanism (dark-coloured forms) in moths in industrialized areas. The number of reported occurrences of melanism has risen steadily since about 1850. About 780 species of the larger moths occur in Britain and over 100 of these now commonly include melanotic forms. Similar changes have been observed in both continental Europe and North America, commonly in association with industrial development, although in some species melanoic individuals do occur in rural areas (Moriarty, 1990). Under strong selection pressure population responses have been observed in a few tens of generations. The

effects of the allele for melanism depends greatly on the whole genome structure, but the precise nature of this selection is still uncertain. Melanism also illustrates the difficulties of producing adequate proof, or disproof, of cause and effect when pollutants are thought to be causing major biological effects (for a detailed discussion, see Moriarty, 1990).

An example of selection by toxic stress is metal resistance in plants. Soils may naturally contain relatively high concentrations of heavy metals or this may result from mining activities. Experiments confirmed that only resistant populations could grow on contaminated soils, but normal and resistant types germinated and grew equally well on uncontaminated soils. However, when the resistant and normal genotypes were grown together on uncontaminated soils, the normal plants grew more successfully than the resistant genotype. Apparently, the adaptation to metals was not without cost. An example is the effect on phosphate uptake in arsenate-tolerant *Holcus lanatus* (Meharg and Macnair, 1990). Metal resistance on contaminated sites is noticeable principally because other common species are absent, but many of the relatively obscure differences that have been described for metal-resistant plants are probably independent adaptations to other environmental gradients that are more or less correlated with the gradient of metal content in the soils (Antonovics *et al.*, 1971). More examples of the cost associated with adaptation to toxic stress are given in Holloway *et al.* (1990).

Another well-known example is the finding in insects of increased resistance to insecticides. This resistance may result from changes in target sensitivity, in penetration, binding and distribution, but most often from improved metabolic detoxification. Generally speaking, the order of importance and frequency of occurrence of types of metabolic resistance follow the overall metabolic activity in insects (Terriere, 1984; Matsumura, 1985): (i) mixed-function oxidases, (ii) esterases and (iii) glutathione S-transferases. In addition, in specific cases other enzyme systems, such as DDT-dehydrochlorinase (DDTase) play a role. Although in some cases major resistance genes have been identified, the characteristic is most often a polygenic phenomenon that can involve tremendous synergistic effects. One of the best examples of this type of interaction can be found in the work of Georghiou (1971). He isolated resistance factors on each chromosome from a multiresistant housefly strain. Whereas individual genes increased the resistance compared with a susceptible strain by factors between 1.5 and 3, the combined action resulted in a 200-fold resistance. This is an extremely important point, showing that with extended accumulation of mutations (either naturally, mutagen-induced or even generated by mutator genes, see below), each resulting in a small or negligible effect, certain combinations may result in a dramatic effect.

The key to understanding gene frequencies and resistance development is to realize that resistance genes can persist for many years once they are selected and established (e.g. in houseflies resistance to DDT and cyclodiene was present even after a 20 year period of no spraying). In Drosophila, DDT resistance depending on high expression of cytochrome P450-dependent xenobiotic metabolizing capacity persisted over 10 years without selection in the laboratory (Frölich and Würgler, 1990). Nevertheless, once the insecticidal pressure is off, the frequencies of resistance alleles may also decline, so that field populations will generally revert to seemingly normal levels; alleles are rarely completely lost. Interestingly, in insect populations that have reverted to normal susceptibility levels after the cessation of pesticide application, the return of resistance is often much quicker once the pressure by the same or a related pesticide spray programme is resumed, e.g. within an integrated pest management scheme. An example is the appearance of a propensity for pyrethroid resistance in places where DDT had been used in the past (Metcalf, 1980). This phenomenon has been explained by the establishment of resistant subpopulations, resulting from recombination of resistance genes still available in the population.

In humans a well appreciated example of selection by environmental stress is the frequency of sickle-cell anaemia (Allison, 1956, 1975). In some parts of Africa up to 40% of the population are heterozygous for the HbS allele and it correlates with the occurrence of the most severe form of malaria. The high allele frequency is maintained because the heterozygous individuals (HbA/HbS) have a selective advantage over both the normal genotypes (HbA/HbA) who may die of malaria, commonly in childhood, and the homozygous sickle-cell genotypes (HbS/HbS) which are anaemic and frequently develop haemolytic crises which often are responsible for death. Malaria is involved in a number of additional human polymorphisms leading to increased heterozygosity levels under its influence, i.e. abnormal haemoglobin E, thalassaemia, and glucose-6-phosphate dehydrogenase deficiency (Livingston, 1971).

Environmental stress as inducer of mutation and recombination

Some rather preliminary evidence is accumulating in the recent literature that *de novo* variation due to recombination and mutation in the broadest sense increases under stress (Parsons, 1987a,b). Hence at stressful moments in evolutionary history when there is a premium on major adaptive shifts, variability of all types may be increased and this could trigger genomic reorganizations in response to rapidly changing environments (McClintock, 1984; Cullis, 1987). There is, however, a major problem in these studies since already a slight increase in stress could lead to lethality or no variation, which means that the interface between high variation and the potential for species elimination is likely to be extremely narrow (Parsons, 1987b). McDonald (1990) published an interesting review covering some of the evidence for adaptive genetic change under environmental stress, involving retroviral insertion or changes in the regulation of gene expression. Hall (1989) presented evidence of increased mutation frequencies in bacterial genes under environmental stress conditions.

In this context the so-called 'mutator' genes should be mentioned. The first mutator mutation detected was present in a wild population of Drosophila (see Mohn and Würgler, 1972). In the meantime, many incidences of mutator mutations in diverse animal and plant species and in microorganisms have been reported. The phenotype of such mutations is, for example, via decreased accuracy of a DNA-polymerase, a constantly increased frequency of 'spontaneous mutations'. Clones of organisms carrying such a mutation will mimic an intensive exposure to mutagens.

Possible consequences of enhanced mutation frequencies by mutagen exposure

As we have seen in the above sections, newly arisen genetic changes, when harmful, will be eliminated quickly from the population without appreciable damage to that population in view of the low frequencies and the reproduction surplus that is usually present. When neutral, mutations may persist and provide a source of variation useful for adaptations needed in the future. Only when mutations confer a selective advantage in connection

with a particular environmental condition will they spread in the population.

Thus, with respect to the possible impact of an *enhancement* of mutation frequencies by mutagen exposure of natural populations, we may conclude that a moderately increased frequency of rare mutations is not likely to have immediate harmful effects. On the contrary, it may speed up adaptation and microevolution in situations of adverse environmental conditions (such as climatic conditions, toxic stresses or even the toxic action of the mutagenic compound itself). It also may facilitate the development of resistance to pesticides and thus reduce their 'economic half-life'. On the other hand, one could think of one particular type of mutation as being harmful at the population level in a relevant manner, i.e. one occurring in a polygenic system affecting a fitness trait such as mating efficiency or reproductive capacity. This type of mutation may be induced at high enough frequencies to be no longer 'a rare event' and at the same time it may affect vital population functions (Ramel, 1983; Kramers *et al.*, 1991).

Also, mutations affecting complex behavioural patterns, as they have been experimentally induced in rats, may be included in this category (Lowery *et al.*, 1990). An important point in fitness traits, in comparison with other quantitative characteristics, is that they are usually optimized, i.e. they show little additive genetic variability (Bulmer, 1989; Roff and Mousseau, 1987). Therefore, a new mutation in a fitness trait is more likely to have a harmful effect.

The induction of genetic effects by genotoxins in *somatic* cells of animals or plants may, as in human beings, give rise to neoplasia. If this occurs after the main reproductive period and does not otherwise interfere with reproduction, this will usually not be critical for the maintenance of the population. On the other hand, the monitoring of genetic changes (that may by themselves be harmless) in somatic cells of organisms in their natural habitat can be applied as indicators of the presence of genotoxins in the environment (see below).

Presence of genotoxins in the environment: the development of biomonitoring systems for use *in situ*

Even if we presume, as suggested above, that an enhanced mutation frequency in natural populations will generally not present a major ecotoxicological problem, there are sufficient reasons left to consider the unlimited release of genotoxins in the environment as an undesirable situation. Therefore, many efforts have been undertaken to develop efficient systems for the detection of genotoxins in air, water and other environmental compartments. These efforts generally have two approaches. The more classical one is to take samples of air particles, water or soil, prepare an organic extract and investigate these in the laboratory using routine genotoxicity assays or chemical analysis. The other is to score for genotoxic effects in animals or plants that can be exposed *in situ*, in their natural environment, i.e. without the need of prior concentration or extraction procedures of the test media (for a recent review, see De Flora *et al.*, 1991).

Many examples are known of the first type. Samples from polluted marine environments have been shown by standard genetic toxicological methods to cause damage to DNA (e.g. Colombatti *et al.*, 1976; Parry *et al.*, 1976; Bayne *et al.*, 1985). Genotoxic components from river and marine sediments collected in the vicinity of Barcelona (Spain) were characterized using mass spectroscopy in combination with the Salmonella/microsome test (Grifoll *et al.*, 1990). In recent studies sediments from a river in an industrialized area in Ohio (USA) and from inshore industrial sites at the Great Lakes polycyclic aromatic hydrocarbons (PAHs) were extracted and their genotoxic potential determined using the Ames test and unscheduled DNA synthesis in rat hepatocytes. The PAHs accounted for most of the genotoxic activity found in polluted river sediments in one study (West *et al.*, 1988); however, in the other, mutagenicity could not be completely related to the degree of contamination by PAHs (Fabacher *et al.*, 1988). A variety of marine animals have been shown to accumulate certain classes of pollutants in their tissues (Chipman, 1972; Roberts, 1976; Grimas, 1979) and many of these organisms, including several important food species, are known to have the metabolic capability for transforming various xenobiotics to mutagens (Payne and Martins, 1980; Bayne *et al.*, 1985). It has recently been shown that PAHs and polychlorinated biphenyls (PCBs) present in sediments are taken up by blue mussel (*Mytilus edulis*). The increased frequency of renal and pancreatic neoplasms and hepatotoxic neoplastic precursor lesions in winter flounder (*Pseudopleuronectes americanus*) feeding on these mussels is a demonstration of the trophic transfer of sediment-bound carcinogens up the food chain (Gardner *et al.*, 1991).

Concerning the second approach, recent literature provides many examples. A micronucleus test using erythrocytes of *Pleurodeles waltl* larvae (Amphibian, Salamandriae) was validated after laboratory exposure to 19 compounds (Fernandez *et al.*, 1989). The induction of chromosome breaks and C-mitoses by methyl mercury chloride (CH_3HgCl) and mercuric chloride ($HgCl_2$) was found in red blood cells of larvae and embryos of *P.waltl* (Zoll *et al.*, 1988). In the same organism free chlorine and monochloramine in the water induced significant frequencies of micronuclei in erythrocytes of larvae and adult animals (Gauthier *et al.*, 1989). Among marine animals the embryos of the tubeworm *Pomatoceros triqueter* (L.) (Serpulidae; Polychaeta) proved to be excellent material for genetic toxicity testing (Bayne *et al.*, 1985). In peripheral erythrocytes of the fish *Heteropneustes fossilis* it has been shown that mitomycin C as well as paper mill effluent induce micronuclei (Das and Nanda, 1986). For a review of the use of fish as biological detectors of the effects of genotoxic agents see Klingerman (1982) and Maddock *et al.* (1986). Using a range of direct- and indirect-acting mutagens, it was demonstrated that the larvae of the sediment-dwelling polychaete *Neanthes arenaceodentata* can be used as the basis for an extremely sensitive *in vivo* assay for measuring chromosomal effects (Pesch *et al.*, 1980, 1981). Pesch's sister chromatid exchange technique has been successfully applied to the adults and larvae of the mussel *M.edulis* (Dixon and Clarke, 1982; Harrison and Jones, 1982). The level of sensitivity exhibited by the cells of these two marine invertebrates for standard mutagens is comparable to that recognized for mammalian cells. Mitomycin C-induced micronuclei in the gills of marine mussels (*M.galloprovincialis*) persist for ~2 months and probably longer. Therefore these mussels appear to be a suitable system to monitor environmental genotoxicants (Majone *et al.*, 1987).

The actual use of biota as monitors of genotoxin pollution in their natural habitats is considered, e.g. in China, using the detection of micronuclei in nucleated erythrocytes from various vertebrates (Pisces, Amphibia, Reptilia and Aves) (Zhang *et al.*, 1984) and sister chromatid exchanges in the lymphocytes of the widely distributed toad *Bufo bufo gargarizans* (Lu *et al.*, 1984). A comparison of mussel (*M.edulis*) embryos in samples originating from clean (Whitesand Bay) and polluted (King's

Dock) sites showed that ~25% of the embryos from the polluted, but only ~8% from the clean water, were aneuploid (having abnormal chromosome numbers) (Dixon, 1982). Field-based investigations were carried out on the influence of general pollution stress and acute hydrocarbon pollution on the incidence of various developmental abnormalities, including some chromosomal aberrations, in the planktonic eggs and larvae of two marine fish species (Longwell, 1976). Her results, supported by the findings of radiation studies (IAEA, 1979), show that the cells of fish, especially the eggs and early developmental stages, are very sensitive to chromosome damage from contact with water-borne mutagens (Bayne *et al.*, 1985). In a study in Yugoslavia Kurelec *et al.* (1989) found no statistically significant differences between the DNA adduct levels of fish from the unpolluted Korana River and the polluted Sava River. They assume that most of the detected DNA modifications are caused by natural factors rather than man-made chemicals.

Plants are sensitive *in situ* detectors of atmospheric mutagens (for a review, see Grant and Zura, 1982). The more common higher plant assays used *in situ* are those using *Tradescantia paludosa* and *Zea mays*, but many other plants such as *Allium cepa, Arabidopsis thaliana, Glycine max, Hordeum vulgare, Lycopersicon escultenum, Vicia faba* and also weed communities are used. The different systems allow the detection of somatic mutations, chromosome aberrations, micronuclei and mitotic recombination, i.e. the whole spectrum of adverse genotoxic effects. Tradescantia systems have also been successfully used to monitor genotoxin pollution of surface waters (Ma, 1989; Ruiz *et al.*, 1989).

A related issue is the occurrence of neoplasia in marine shellfish and fish in European as well as North American waters, for which exposure to genotoxins has been advocated as the cause. The evidence concerning this subject has been reviewed recently (GESAMP, 1991). An interesting aspect is that in fish tumours the same types of oncogenes are activated as in neoplasms of mammals and man (see, e.g. Chang *et al.*, 1991; Mangold *et al.*, 1991). In laboratory experiments, exposure of eye-stage rainbow trout (*Salmo gairdneri*) embryos showed that the genotoxic rodent carcinogens aflatoxin B_1, benzo[*a*]pyrene, dimethylnitrosamine and *N*-methyl-*N'*-nitrosoguanidine induced well-differentiated hepatocellular carcinomas (Maccubbin and Black, 1986).

Fate of genetically altered organisms in the environment

Related to the question of the fate of newly-induced mutations in natural populations is the problem of the release of new genetic variants into a natural environment. This is a hot issue with respect to genetically-engineered strains, but an older variant of the same problem is the intended or unintended introduction of varieties or even species into new areas with sometimes dramatic consequences for existing ecosystems (rabbits in Australia, Dutch elm disease in America, rats in Madagascar, etc; Elton, 1958). The critical point with organisms released into a new environment is whether or not the 'new' genotype has an advantage over its competitors or can fill an unoccupied niche. These conditions are obviously rarely met and presumably the great majority of such transplants were failures. Only the successful ones became known.

In the field of microorganisms, we know that quantities of bacteria are released into the environment continuously in sewage and that millions of hectares of land are inoculated with Rhizobium each year to improve the growth of leguminous crops. One important lesson from such studies in this field (J.E.Beringer, University of Bristol; see Glover, 1988) is that introduced strains do not usually compete successfully for nodule formation where indigenous rhizobia are well established. Over the past few years, rhizobia carrying transposable genetic elements conferring drug resistance have been released to study the fate of the genes carried in their plasmids. The *lacZY* genes of *Escherichia coli* delivered to fluorescent Pseudomonads by an engineered *Tn7–lac* element are a highly effective tracker-gene system and were released in a test approved by the Environmental Protection Agency (EPA) in the US (G.F.Barry, Monsanto, St Louis; see Glover, 1988). Both vertical and horizontal movements of the Pseudomonads away from the site of inoculation were monitored and found to be negligible.

Recent work at a deep ocean dump site off the coast of Puerto Rico has shown that changes in the microbial populations of the waters receiving foreign bacteria can be detected, i.e. changes in bacterial community structure, over and above seasonal effects, have been documented (Colwell and Grimes, 1986). Microbial impact of the dumping wastes occurs at three levels that can be measured. These include the initial effects at the time of dumping, followed by sustained community structural changes and, finally, genetic modification of the natural population evidenced by increased incidence of plasmids. The ocean dumping studies were augmented by examination of the incidence of plasmids in bacteria isolated from samples collected at other locations in the Atlantic Ocean, including outfall samples collected at Barceloneta, Puerto Rico, offshore samples collected at an outfall of Ocean City, Maryland, and a clean unpolluted site. The incidence of plasmids could be significantly and dramatically related to influx of sewage. Thus, environmental changes already occur as a result of entrance of allochthonous material into the marine environment (Cairns and Pratt, 1986).

Examples of horizontal transfer of genetic material between species may be detectable in cases in which experimental *viruses* are introduced into the environment. They may give us some ideas how mutationally altered types, including those resulting from the environmental contamination with genotoxins, might spread. A different situation from that observed with bacteria developed in an experiment with a vaccinia-rabies recombinant virus. No approval from appropriate authorities in Argentina was obtained for a field trial in Azul conducted by investigators from the Wistar Institute (Philadelphia) and the Pan American Health Organization in September 1986 (see Palca, 1986; Glover, 1988). The experiment involved the testing of a vaccinia-rabies recombinant virus vaccine in cattle. It was not so much the experiment itself that was the subject of criticism, but the way it was planned and implemented. Preliminary results of monitoring since the experiment, now interrupted by the Argentinian authorities, show that all vaccinated and contact animals were seroconverted 6 months after the start of the experiment. Analysis of blood samples from three of the four local animal caretakers indicate that one was seroconverted for rabies virus antibodies. The recombinant virus appears, therefore, to have passed from vaccinated animals to all of their animal contacts and to humans involved in handling and milking these animals (J.La Torre, Centro de Virologica Animal Serrano, Argentina; see Glover, 1988). This spreading seems not to be a general problem with viruses since in November 1986 a team from the Oregon State University in Corvallis had better success testing its modified vaccinia virus. A field trial for a vaccine for Sindbis virus had been carried out over a year in New Zealand. Calves, sheep and chickens were vaccinated in the study. None developed disease or transmitted the virus to unvaccinated animals (Palca, 1986).

Concluding remarks

In the preceding sections the possible consequences of enhanced mutation frequencies in natural populations, as they might occur by exposure to mutagenic agents, have been considered against the background of the significance of mutation and selection in the dynamics of populations and species. It was concluded that mutations in plants or animals are not necessarily bad events, as they are considered in human beings; when they do not adversely affect fitness characteristics of the population, they may increase genetic variation which may be advantageous in stressful environmental conditions. These may be advantages for the species in question but not for its neighbours. Examples are (i) the induction of (pesticide) resistance, (ii) the increased virulence of pathogens, (iii) alterations of host ranges of pathogenic forms or the appearance of new virus types and (iv) subtle changes in parasite–host or predator–prey relationships, in other words, increased instability in ecosystems.

Having said all this, what are the main arguments left against releasing large amounts of genotoxins into the natural environment? In qualitative terms, these are:

(i) Upon massive exposure (implying large increases in mutation frequency) there may be genetically-based effects on fitness, as well as straightforward toxic effects. The latter may include toxic effects on reproductive cells (which can be expected for genotoxic compounds) that could severely affect reproduction (Kramers *et al.*, 1991).

(ii) More modest increases in mutation frequency could work out 'positively', by giving a population a better chance to escape stressful situations (at the same time the anticipated reduced economic lifetime of pesticides could be judged negatively), or 'negatively', by enhancing instability in ecosystems.

(iii) Finally, the uncontrolled presence of genotoxins in all compartmens of the natural environment carries the possibility of human exposure by unknown compounds from unknown sources and routes. This is an unwanted situation. In particular, this point justifies the monitoring of the environment for the presence of genotoxins.

As to the quantitative side, we have as yet no substantial information that would allow us to perform a specific risk analysis with respect to the effects of genotoxins in the environment. It seems reasonable to suspect that, especially in the situation of moderate exposure [as indicated above under (ii)], the variety of ecosystems exposed and the variety of possible outcomes in each specific situation refutes any quantitative approach. On the other hand, the fact that (for the time being mainly on the grounds of potential human risk) monitoring for genotoxins in air, water, effluents, etc., is being carried out, requires a quantitative view on the outcome of these measurements.

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